Reference: *Biol. Bull.* **163:** 453–464. (December 1982)

MALE PRONUCLEAR DEVELOPMENT IN STARFISH OOCYTES TREATED WITH 1-METHYLADENINE

FRANK J. LONGO[1,*] AND ALLEN W. SCHUETZ[2]

[1]*Department of Anatomy, University of Iowa, Iowa City, IA 52242,* [2]*Department of Population Dynamics, John Hopkins School of Hygiene, Baltimore, MD 21205, and the* [1,2]*Marine Biological Laboratory, Woods Hole, MA 02543*

Abstract

Light and electron microscopic observations were carried out in order to examine the relation between male pronuclear development and the state of "cytoplasmic maturation" acquired by starfish oocytes under the influence of 1-methyladenine (1-MA). Alterations were not apparent in the germinal vesicles or incorporated sperm nuclei of inseminated immature *Asterias* eggs for up to 5 hours in the absence of 1-MA. With the addition of 1-MA dramatic changes occurred in the germinal vesicle and ooplasmic region associated with incorporated sperm nuclei. These were followed by alterations in the sperm nucleus leading to the development of a male pronucleus. Pronuclear development in *Asterias* eggs inseminated at the germinal vesicle stage and then treated with 1-MA differed from that described for other organisms. Aside from the dilation of its perinuclear cisterna, the sperm nuclear envelope persisted intact throughout development. Dispersion of condensed chromatin occurred simultaneously throughout the whole of the sperm nucleus. These results suggest that factors necessary for pronuclear development do not exist in the ooplasm of immature starfish oocytes but arise following dispersal of germinal vesicle contents into the cytoplasm.

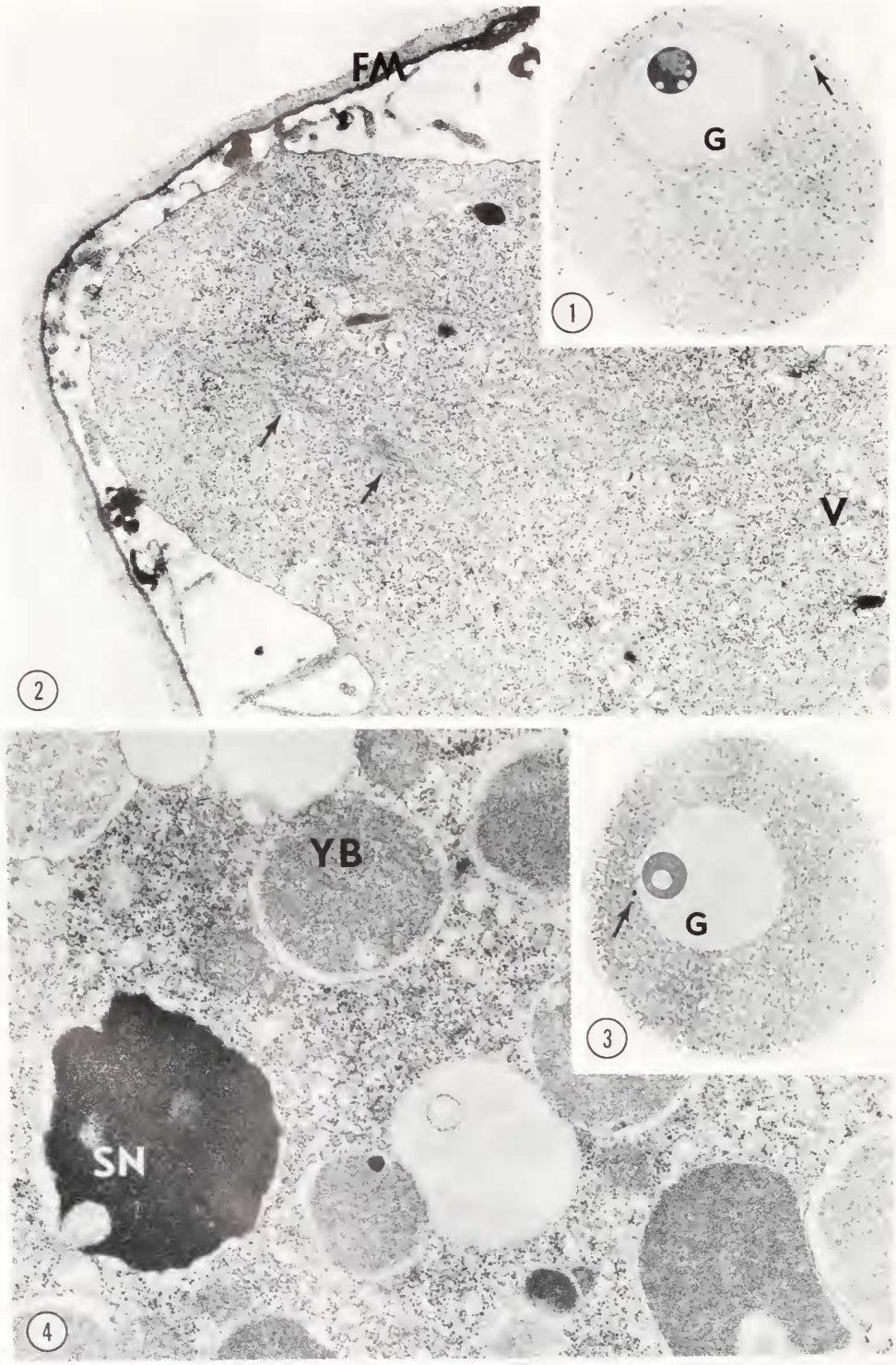
Introduction

The eggs of most animals initiate meiotic maturation prior to ovulation, become arrested at a specific stage of meiosis, and resume maturation after insemination. Although eggs may be experimentally manipulated to ferilize prematurely, *i.e.*, at an earlier stage of meiosis, investigations with the ova of a number of different organisms have indicated that germinal vesicle breakdown is a prerequisite in establishing a condition of cytoplasmic maturation which supports the transformation of a fertilizing spermatozoon into a male pronucleus (Skoblina, 1974, 1976; Hirai, 1976; Katagiri and Moriya, 1976; Thadani, 1979; Balakier and Tarkowski, 1980; Hirai *et al.*, 1981).

Germinal vesicle-intact (immature) starfish oocytes, induced to mature by ovarian hormone (1-methyladenine; 1-MA), develop normally when fertilized (Kanatani and Shirai, 1967; Schuetz and Biggers, 1967; Kanatani *et al.*, 1969). Germinal vesicle-intact oocytes may also be inseminated and subsequently treated with 1-MA to induce germinal vesicle breakdown (Cayer *et al.*, 1975; Schuetz, 1975; Schuetz and Longo, 1981). That the onset of germinal vesicle breakdown can be controlled by exogenous substances in starfish eggs provides a means of studying nucleocytoplasmic interactions during male pronuclear development and the role of 1-MA in fertilization and the onset of development.

Received 24 May 1982; accepted 7 September 1982.

* Author to whom correspondence should be addressed (at Univ. of Iowa).


FM
G
1
V
2
YB
G
3
SN
4

The present light and electron microscopic study examines the relation between processes of fertilization and the state of "cytoplasmic maturation" acquired by oocytes under the influence of 1-MA. For this purpose observations were designed to analyze sperm-egg interactions in fertilized, intact germinal vesicle oocytes of the starfish, *Asterias forbesi*, before and after exposure to 1-MA. Brief accounts of these observations have been published previously (Schuetz and Longo, 1979, 1981).

Materials and Methods

Germinal vesicle-intact oocytes were obtained from ripe *Asterias forbesi* as described by Longo *et al.* (1982). Oocytes were fertilized with a 0.1% suspension of sperm and samples were fixed at varying intervals up to 5 hours postinsemination. At forty-five minutes postinsemination a portion of this suspension was treated with 1-MA (1 μg/ml) and samples were taken at varying intervals and prepared for light and electron microscopy as previously described (Longo *et al.*, 1982). A second suspension of oocytes was treated with 1-MA (1 μg/ml) to induce meiotic maturation; these oocytes are referred to as maturing ova. Approximately 30 minutes later the eggs were fertilized and sampled at periodic intervals up to 90 minutes postinsemination.

Results

Within 5 minutes of gamete mixing, sperm were seen within immature and maturing ova, usually located at the base of the fertilization cone (Fig. 1). By this time the cortical granule reaction was completed and a fertilization membrane surrounded the inseminated egg (Figs. 1, 2). All of the immature starfish oocytes examined in this study were polyspermic (Schuetz and Longo, 1981); eggs that had undergone germinal vesicle breakdown prior to insemination were monospermic. A more detailed ultrastructural account of male and female pronuclear development and association in monospermic, maturing *Asterias* eggs is the subject of a subsequent report; light microscopic observations have been presented by Hirai *et al.* (1981).

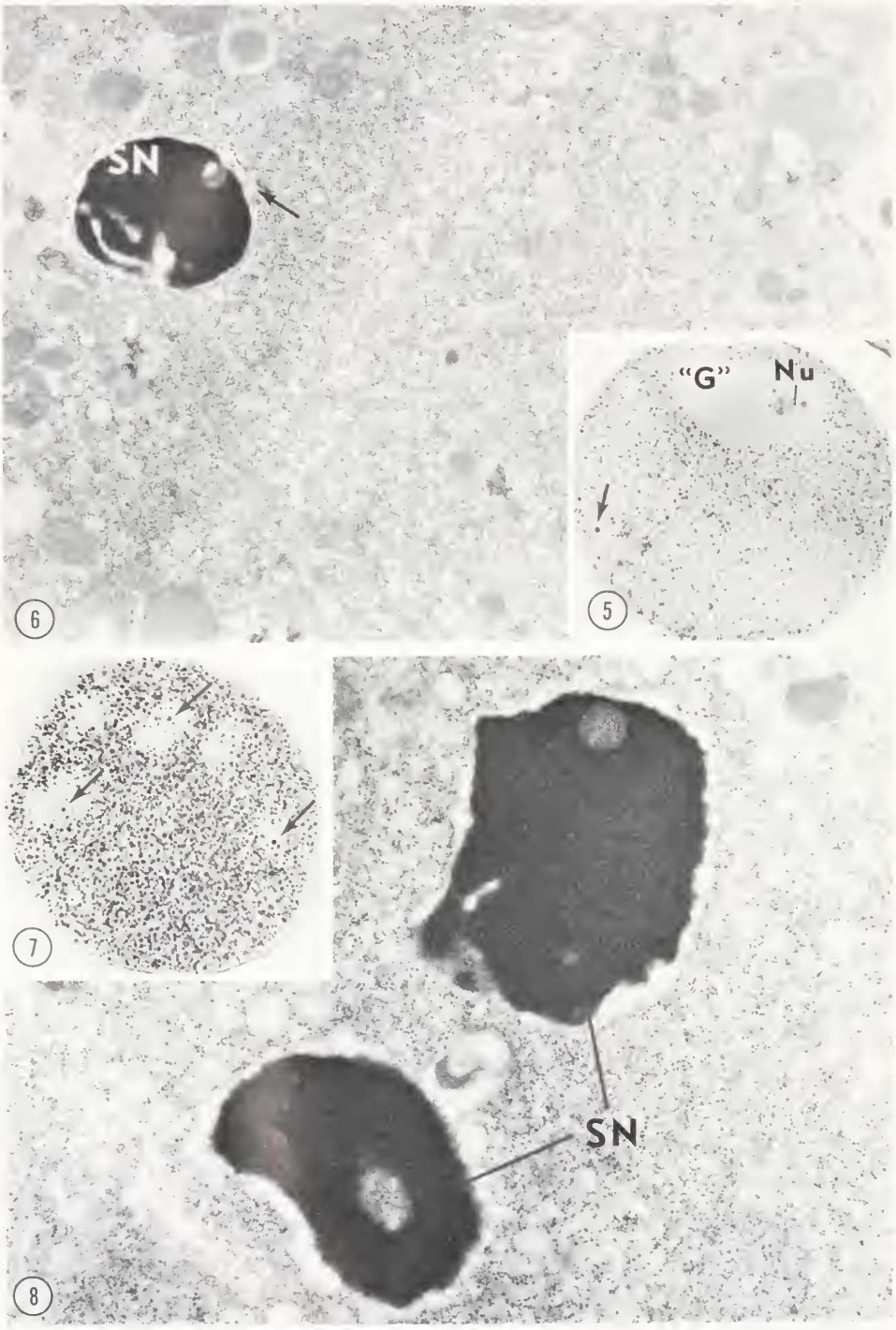
The fertilization cone, through which the sperm nucleus passed during its incorporation, was larger in germinal vesicle oocytes than in maturing eggs. When the fertilization cone achieved its maximum dimensions (at about 5 minutes postinsemination) in germinal vesicle-intact eggs it extended approximately 2.5 μm from the oocyte surface and was about 1 μm in diameter at its base. Morphologically it contained a granular substance and fascicles of microfilaments. Along its proximal aspect were numerous vesicles (Fig. 2).

FIGURE 1. Immature *Asterias* oocyte, 5 minutes postinsemination. At the base of the fertilization cone is an incorporated sperm nucleus (arrow). G, germinal vesicle containing a nucleolus. ×1,000.

FIGURE 2. Fertilization cone of an immature oocyte, containing ground substance and fascicles of microfilaments (arrows), 5 minutes postinsemination. Along the base of the fertilization cone are aggregations of vesicles (V). FM, fertilization membrane. ×14,000.

FIGURE 3. Immature oocyte, 30 minutes postinsemination. An incorporated sperm nucleus is depicted at the arrow. The germinal vesicle (G) is structurally similar to those observed in unfertilized oocytes. ×1,200.

FIGURE 4. Sperm nucleus (SN) incorporated into an immature oocyte. In inseminated, immature oocytes, ooplasmic organelles surround the incorporated sperm nucleus and a specialized region, lacking organelles and characteristic of fertilized eggs treated with 1-MA, is not observed. YB, yolk bodies. ×10,000.


SN
"G"
Nu
SN
6
5
7
8

Sperm nuclei incorporated into germinal vesicle eggs initially were found within the cortex; with time (*i.e.*, by 30 minutes postinsemination) however, they were distributed throughout the cytoplasm without any apparent relation to ooplasmic components (Fig. 3). The cytoplasmic area which surrounded incorporated sperm nuclei was unspecialized in that it contained the same content of organelles and inclusions as observed in other portions of the oocyte (Fig. 4). When inseminated oocytes were maintained at 20°C with gentle agitation, sperm nuclei remained unchanged for up to 5 hours. Throughout this period the sperm nuclear envelope remained intact and the condensed sperm chromatin showed no signs of dispersion (Fig. 4). Moreover, the process of insemination and the presence of incorporated sperm did not appear to have any morphological effect on the germinal vesicle (Fig. 3).

By 15 minutes following the addition of 1-MA profound, structural alterations were apparent in the germinal vesicle, as well as with the ooplasm surrounding incorporated sperm nuclei. The cytoplasmic area surrounding the sperm nucleus became clear of organelles, such as yolk bodies and mitochondria, and within this region accumulated endoplasmic reticulum and ground substance (Figs. 5, 6). This cytoplasmic area enlarged to greater than 12 μm in diameter before morphological changes were noted within sperm nuclei (Fig. 7). In many polyspermic eggs more than one sperm nucleus was associated with such a specialized area (Fig. 8).

By 30 minutes following the addition of 1-MA, changes in incorporated sperm nuclei had become apparent. Dilation of the perinuclear cisternae was pronounced, and alterations in the density and composition of the condensed chromatin occurred. The actual disruption or removal of the sperm nuclear envelope, similar to that seen in other species (Longo, 1973), was not observed, and how this membranous structure was modified to accompany the expansion of the paternally derived chromatin was not obvious (Fig. 9).

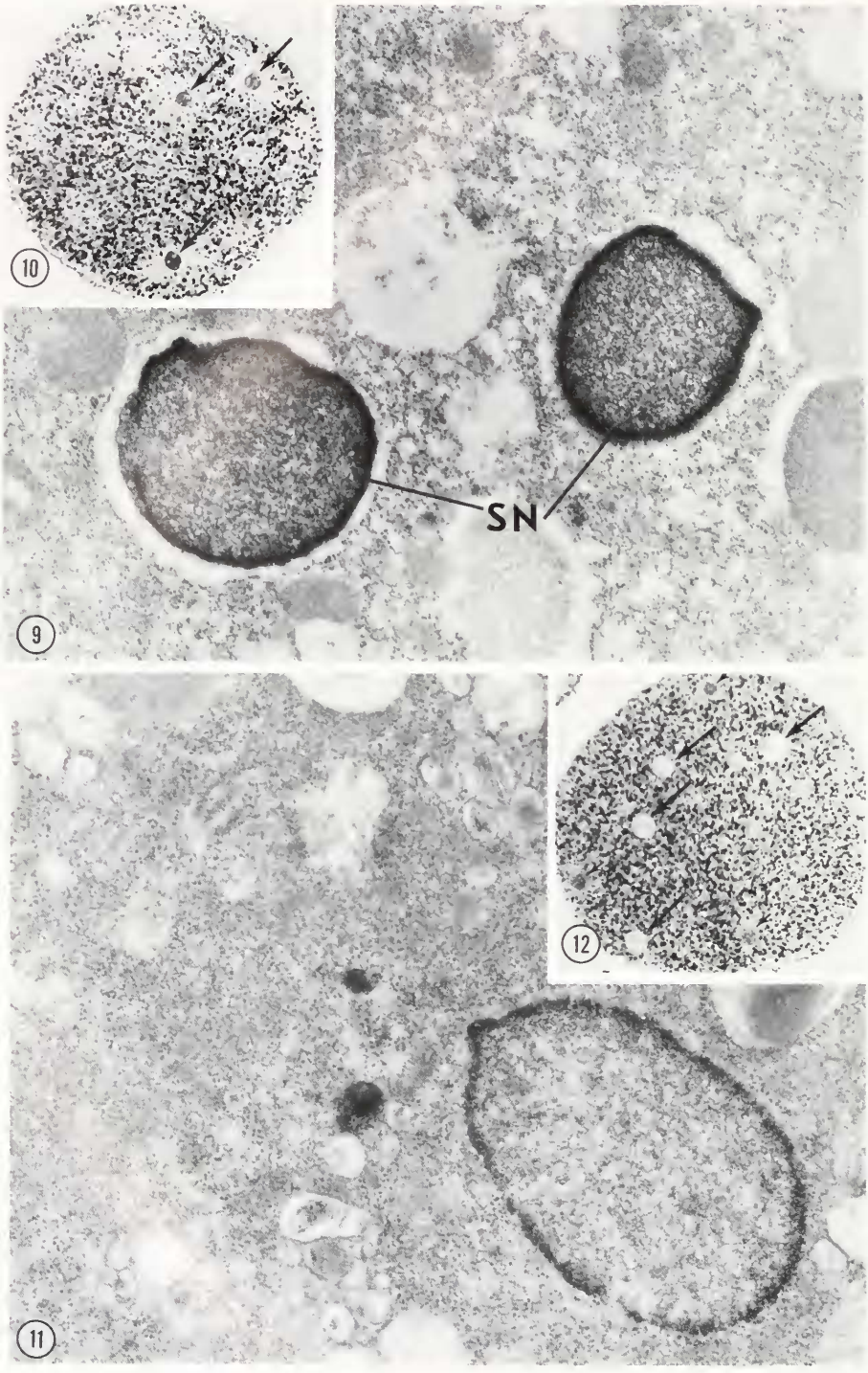
Chromatin dispersion appeared to differ from that described for pronuclear development in zygotes of other species (*cf.* Longo, 1973, 1981). The condensed chromatin gradually transformed from a dense substance to a dispersed, filamentous mass (Figs. 9–12). These changes occurred simultaneously throughout the sperm nucleus except for that material bordering the inner margin of the nuclear envelope (Figs. 9, 11). The condensed chromatin lining the periphery of the sperm nucleus remained unchanged until late in the development of the male pronucleus (60 to 90 minutes after the addition of 1-MA).

FIGURE 5. Inseminated, immature oocyte, 15 minutes after the addition of 1-MA. The germinal vesicle is breaking down ("G"). An incorporated sperm nucleus, surrounded by a "clear" cytoplasmic area is shown at the arrow. Nu, portion of the disrupting nucleolus. ×1,000.

FIGURE 6. Sperm nucleus (SN) incorporated into an immature oocyte that was subsequently treated with 1-MA (15 minutes after the addition of 1-MA). A cytoplasmic region, relatively devoid of organelles, is associated with the sperm nucleus. The arrow depicts a portion of the sperm nuclear envelope in which the perinuclear cisterna is dilated. ×15,000.

FIGURE 7. Inseminated, immature oocyte subsequently treated with 1-MA. Sperm nuclei which are surrounded by an area relatively free of cytoplasmic organelles are shown at the arrows. Sample fixed 30 minutes after the addition of 1-MA. ×1,000.

FIGURE 8. Two sperm nuclei (SN) of a polyspermic, immature oocyte treated with 1-MA for 15 minutes. Although the condensed chromatin does not show any recognizable changes when compared to oocytes not treated with 1-MA, the perinuclear cisternae of the sperm nuclear envelopes are dilated. ×20,000.


10
SN
9
12
11

Changes in sperm nuclear morphology were not uniform as there was considerable asynchrony in pronuclear development in fertilized oocytes treated with 1-MA (Fig. 12). This asynchrony appeared to be a temporal one, since eventually all incorporated sperm nuclei developed into male pronuclei. The relation of this asynchrony to a specific location within the zygote, *e.g.*, the site of germinal vesicle breakdown or sperm entry, was not apparent.

One to 2 hours following the addition of 1-MA, male pronuclei were observed with well-dispersed chromatin and continuous nuclear envelopes (Fig. 13). The nuclear envelope did not demonstrate the dilations of the perinuclear cisternae characteristic of metamorphosing incorporated sperm nuclei. Internally, clear areas, surrounded by a granular nucleoplasm, filled the male pronucleus. Nucleoli composed of a dense granular material also appeared within developed male pronuclei. The male pronuclei continued to enlarge and by 120 minutes following the initiation of pronuclear development measured 5 to 10 μm in diameter. Large, irregular male pronuclei were also observed within polyspermic zygotes suggesting that the pronuclei fused with one another (Fig. 14).

Following the completion of male pronuclear development the cytoplasmic areas, characteristically associated with transforming sperm nuclei, were greatly reduced in size relative to the size of the male pronucleus. Male pronuclei were surrounded by cytoplasmic areas containing ground substance and some endoplasmic reticulum. This morphology persisted for approximately 2.5 hours after the addition of 1-MA, at which time the pronuclei demonstrated changes characteristic of prophase, *i.e.*, chromosome condensation and nuclear envelope breakdown. Concomitant with these changes spindles were formed in association with the condensing paternally derived chromosomes; the numerous mitotic figures that were produced were observed throughout the fertilized egg (Fig. 15). Of the inseminated immature oocytes treated with 1-MA for 4 hours, less than 10% cleaved into what appeared to be "normal" embryos. Most underwent a succession of divisions such that "morula"-like structures, consisting of blastomeres of different sizes, were produced (Fig. 16).

Discussion

The microscopic observations presented here document changes induced by 1-MA treatment on sperm nuclei incorporated into immature *Asterias* eggs. Morphological changes in the germinal vesicle or incorporated sperm nuclei were not apparent for up to 5 h in the absence of 1-MA. With the addition of 1-MA dramatic

---

FIGURE 9. Sperm nuclei (SN) of an immature oocyte treated with 1-MA for 30 minutes. The condensed sperm chromatin is dispersing except for that which lines the nuclear envelope. ×28,000.

FIGURE 10. Transforming sperm nuclei (arrows) in an immature oocyte treated with 1-MA for 45 minutes. Around each of the developing pronuclei is a specialized cytoplasmic region lacking organelles. ×1,300.

FIGURE 11. Transforming sperm nucleus in an immature oocyte treated with 1-MA for 45 minutes. The condensed chromatin is dispersed except for that located along the periphery of the transforming sperm nucleus. ×29,000.

FIGURE 12. Transforming sperm nuclei at early (small arrows) and later (large arrows) stages of pronuclear development. The earlier stages are distinguished by dense chromatin. Notice that the more developed male pronuclei, *i.e.*, those with the more dispersed chromatin, lack the specialized cytoplasmic areas characteristic of earlier stages. ×1,200.

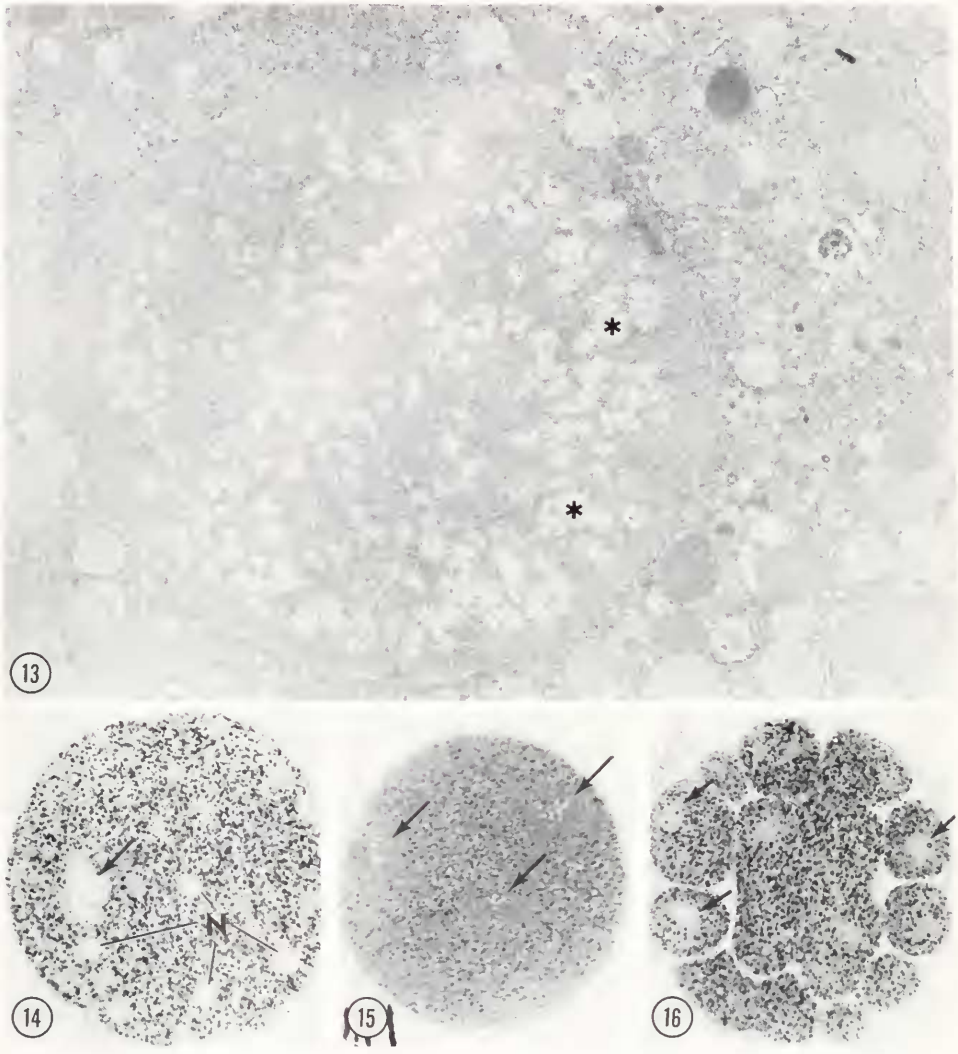


FIGURE 13. Male pronucleus in a fertilized oocyte treated with 1-MA for 60 minutes. A nuclear envelope defines the border of the pronucleus which is surrounded by cytoplasm containing organelles. The clear areas within the pronucleus (*) containing some filamentous material represent areas of chromatin that were extracted by the preparative methods employed. ×14,000.

FIGURE 14. Multiple male pronuclei (N) in a polyspermic oocyte 120 minutes after the addition of 1-MA. The larger, irregular nuclear mass at the arrow appears to be derived from the fusion of a number of pronuclei. The specialized cytoplasmic areas associated with the pronuclei are greatly reduced in size relative to those observed in earlier specimens. ×1,300.

FIGURE 15. Chromosomal masses (arrows) associated with developing spindles and derived from male pronuclei. Specimen prepared 150 minutes after the addition of 1-MA. ×1,200.

FIGURE 16. Inseminated, immature oocyte that has undergone multiple cleavages to form a "morula"-like structure (incubated with 1-MA for 4 hours). The arrows point to nuclei of the blastomeres. ×1,000.

TABLE I

Temporal relation of meiotic maturation, sperm aster morphogenesis, and male pronuclear development in fertilized immature Asterias *oocytes (germinal vesicle intact) subsequently treated with 1-MA.*

Process	Time after addition of 1-MA (minutes)			
	0	15	30	60–120
Meiosis*	Intact germinal vesicle	Initiation of germinal vesicle breakdown (plication of surface of germinal vesicle)	Germinal vesicle breakdown (disappearance of nuclear envelope and nucleolus)	Development of meiotic spindle and polar body formation
Sperm aster morhogenesis	Not present	Present	Increase in size	Relative reduction in size
Sperm nucleus transformation	Sperm nucleus unchanged	Sperm nucleus unchanged	Dilation of sperm nuclear envelope, chromatin decondensation	Completed male pronucleus

* Taken from Longo *et al.* (1982).

changes were first noted in the germinal vesicle and cytoplasmic region associated with the sperm nucleus. These were followed by alterations in the sperm nucleus leading to the formation of a male pronucleus. Similar results, at the light microscopic level of observation with oocytes of *Asterina pectinifera*, have been reported (Hirai *et al.*, 1981).

The structural reorganization of the cytoplasmic area in association with the sperm nucleus was unexpected as morphologically similar specializations, *e.g.*, asters, are usually preceded by the initiation of pronuclear development in the zygotes of other organisms studied to date (Longo, 1973). The observation that these regions developed only in conjunction with incorporated sperm nuclei suggests that a sperm-derived component (*e.g.*, centrioles) is involved in their formation, possibly as an organizing center. The association of these specialized cytoplasmic regions with sperm nuclei and the fact that they are reminiscent of structures earlier microscopists (Wilson, 1925; *cf.* Hirai *et al.*, 1981) referred to as sperm asters, prompts us to refer to them in a similar manner.

The relation of asters and their development to germinal vesicle breakdown is controversial (*cf.* Masui and Clarke, 1979). The absence of asters in fertilized immature starfish eggs and their development in association with 1-MA-induced meiotic maturation as demonstrated herein suggests that aster formation is related to germinal vesicle breakdown (*cf.* also Franklin, 1965; Longo, 1978) and is supported by investigations in which cellular components, such as basal bodies, initiate the development of asters when injected into mature but not immature amphibian eggs (Heidemann and Kirschner, 1975). On the other hand, enucleation experiments with amphibian eggs have suggested that aster formation is independent of a contribution of germinal vesicle materials (Katagiri, 1974; Skoblina, 1974, 1976).

This and previous studies implicate the germinal vesicle as a source of factors necessary for the transformation of the spermatozoon into a male pronucleus in

starfish (Hirai, 1976; Hirai *et al.*, 1981; Schuetz and Longo, 1981). Similar results indicating the control of nuclear activity via factors that arise from or appear in concert with germinal vesicle breakdown have been described (Dettlaff *et al.*, 1964; Niwa and Chang, 1975; Usui and Yanagimachi, 1976; Longo, 1978; Balakier and Tarkowski, 1980; Hylander *et al.*, 1981). The failure of male pronuclear development in enucleate amphibian eggs supports this speculation (Katagiri and Moriya, 1976; Skoblina, 1976). Whether this requirement is the result of specific germinal vesicle factors or arises from nucleo-cytoplasmic interactions following germinal vesicle breakdown is unclear (Kishimoto *et al.*, 1981).

Although the present study of male pronuclear development in *Asterias* employed polyspermic, immature oocytes, it is noteworthy that the transformation of the sperm nucleus in this particular system differed from that described for other species (Longo, 1973). Such differences include the retention of the sperm nuclear envelope, the simultaneous dispersion of chromatin throughout the sperm nucleus, and the formation of the male pronuclear envelope. Changes in the sperm nuclear envelope were not readily apparent in fertilized, immature *Asterias* eggs treated with 1-MA. This membranous structure did not appear to break down by a process of vesiculation as demonstrated in zygotes of many species examined thus far (Longo, 1973, 1981). Aside from the dilation of its perinuclear cisterna, the sperm nuclear envelope persisted intact throughout pronuclear development. This and the dramatic increase in nuclear volume during pronuclear development raises questions as to how the membrane comprising the nuclear envelope is augmented to accommodate the increase in chromatin dispersion. The dilated perinuclear cisternae characteristic of sperm nuclei within *Asterias* oocytes treated with 1-MA may be a manifestation of this augmentation. In addition, vesicles were occasionally observed adjacent to the surface of the developing male pronucleus (F. J. Longo, personal observations). Although we have not been able to document such an event, it is possible that these vesicles fuse with and augment the existing sperm nuclear envelope.

Dispersion of the condensed sperm chromatin in *Asterias* oocytes differed from that described for sea urchins, where decondensation was initiated along the periphery of the sperm nucleus and progressively appeared more centrad. The pattern observed in *Asterias*, *i.e.*, where dispersion occurred simultaneously throughout the whole of the sperm nucleus, is similar to that described for *Barnea*, *Gallus*, and the hamster (Pasteels, 1963; Okamura and Nishiyama, 1978; Longo and So, 1982).

It has been suggested that the asynchrony in pronuclear development, characteristic of polyspermic *Asterias* oocytes treated with 1-MA, may be related to the proximity of sperm nuclei with the germinal vesicle (Schuetz and Longo, 1981). This spacial relation may be involved, but the observations made during the course of this study, where the extent of pronuclear development was not always correlated with the site of germinal vesicle breakdown, suggest that other factors may have a bearing as well. Previous investigations with mammalian eggs have shown that the degree of polyspermy has a profound influence on pronuclear development (Hunter, 1967; Hirao and Yanagimachi, 1979; Witkowska, 1981). In these studies the number of sperm developing into male pronuclei was inversely related to the degree of polyspermy; some sperm nuclei metamorphosed into male pronuclei while the remainder were delayed at an earlier stage of pronuclear development. These results suggest that in polyspermic eggs competition occurs among sperm nuclei for materials responsible for male pronuclear development. That eventually all sperm nuclei develop into male pronuclei in *Asterias* eggs indicates that the inhibition of pronuclear development is not complete but rather a slowing down of sperm nuclear transformations.

Samples of inseminated oocytes subsequently treated with 1-MA and examined just prior to cleavage, contained large nuclei which appeared to be brought about by a fusion of the pronuclei. The presence of large irregular-shaped nuclei as shown in Figure 14 is suggestive of such a process; pronuclear fusion has also been described in fertilized, immature *Asterina* oocytes treated with 1-MA (Hirai *et al.*, 1981). Eventually all of the nuclei entered mitosis forming what appeared to be individual spindles. Similar results have also been reported for the eggs of other organisms (*cf.* Wilson, 1925; Elinson, 1977). Presumably as a result of the numerous mitotic apparatuses present, the zygote is induced to undergo multiple cleavages (Rappaport, 1971, 1975), such that a morula-like structure is produced. These embryos fail to give rise to normal larvae; they eventually degenerate due presumably to an unbalanced genome.

The microscopic observations presented here further illustrate some of the complex hormonal, cytoplasmic and nuclear interactions that occur during egg maturation and fertilization in *Asterias* and that proper synchronization of these events is crucial for normal development. The temporal relation of meiosis, sperm aster morphogenesis, and male pronuclear development in fertilized, immature *Asterias forbesi* oocytes subsequently treated with 1-MA is outlined in Table I; a similar relation has been described for *Asterina pectinifera* (Hirai *et al.*, 1981).

ACKNOWLEDGMENTS

Portions of this investigation were supported by funds from the NSF and the NIH (HD070401-05). Appreciation is expressed to Leslee Miller, Joyce Kline, and Frederick So for their assistance.

LITERATURE CITED

BALAKIER, H., AND A. K. TARKOWSKI. 1980. The role of germinal vesicle karyoplasm in the development of male pronucleus in the mouse. *Exp. Cell Res.* **128:** 79–85.

CAYER, M. L., T KISHIMOTO, AND H. KANATANI. 1975. Formation of the fertilization membrane by insemination of immature starfish oocytes pretreated with calcium-free seawater. *Dev. Growth Differ.* **17:** 119–125.

DETTLAFF, T. A., L. A. NIKITINA, AND O. G. STROEVA. 1964. The role of the germinal vesicle of oocyte maturation in anurans as revealed by the removal and transplantation of nuclei. *J. Embryol. Exp. Morphol.* **12:** 851–873.

ELINSON, R. P. 1977. Fertilization of immature frog eggs: cleavage and development following subsequent activation. *J. Embryol. Exp. Morphol.* **37:** 187–201.

FRANKLIN, L. E. 1965. Morphology of gamete membrane fusion and of sperm entry into oocytes of the sea urchin. *J. Cell Biol.* **25:** 81–100.

HEIDEMANN, S. R., AND M. V. KIRSCHNER. 1975. Aster formation in eggs of *Xenopus laevis*: induction by isolated basal bodies. *J. Cell Biol.* **67:** 105–117.

HIRAI, S. 1976. The role of germinal vesicle material on the swelling of sperm nuceli penetrated in immature oocytes of the starfish. *Bull. Mar. Biol. Stn. Asamushi* **15:** 165–171.

HIRAI, S., Y. NAGAHAMA, T. KISHIMOTO, AND H. KANATANI. 1981. Cytoplasmic maturity revealed by the structural changes in incorporated spermatozoon during the course of starfish oocyte development. *Dev. Growth Differ.* **23:** 465–478.

HIRAO, Y., AND R. YANAGIMACHI. 1979. Development of pronuclei in polyspermic eggs of the golden hamster. *Zool. Mag. (Tokyo)* **88:** 24–33.

HUNTER, R. H. F. 1967. Polyspermic fertilization in pigs during the luteal phase of the estrous cycle. *J. Exp. Zool.* **165:** 451–460.

HYLANDER, B. L., J. ANSTROM, AND R. G. SUMMERS. 1981. Premature sperm incorporation into the primary oocyte of the polychaete *Pectinaria*: male pronuclear formation and oocyte maturation. *Dev. Biol.* **82:** 382–387.

KANATANI, H., AND H. SHIRAI. 1967. *In vitro* production of meiosis inducing substance by nerve extract in ovary of starfish. *Nature* **216:** 284–286.

KANATANI, H., H. SHIRAI, K. NAKANISHI, AND T. KUROKAWA. 1969. Isolation and identification of meiosis inducing substance in starfish *Asterias amurensis*. *Nature* **221:** 273–274.

KATAGIRI, C. 1974. A high frequency of fertilization in premature and mature coelomic toad eggs after enzymic removal of vitelline membrane. *J. Embryol. Exp. Morphol.* **31:** 573–587.

KATAGIRI, C., AND M. MORIYA. 1976. Spermatozoan response to the toad egg matured after removal of germinal vesicle. *Dev. Biol.* **50:** 235–241.

KISHIMOTO, T., S. HIRAI, AND H. KANATANI. 1981. Role of germinal vesicle material in producing maturation-promoting factor in starfish oocyte. *Dev. Biol.* **81:** 177–181.

LONGO, F. J. 1973. Fertilization: A comparative ultrastructural review. *Biol. Reprod.* **9:** 149–215.

LONGO, F. J. 1978. Insemination of immature sea urchin (*Arbacia punctulata*) eggs. *Dev. Biol.* **62:** 271–291.

LONGO, F. J. 1981. Regulation of pronuclear development. Pp. 529–557 in *Bioregulators of Reproduction*. G. Jagiello and H. J. Vogel, Eds. Academic Press. N. Y.

LONGO, F. J., AND F. SO. 1982. Morphogenesis of sperm nuclei incorporated into aged and unaged hamster eggs. *J. Andrology* (in press).

LONGO, F. J., F. SO, AND A. W. SCHUETZ. 1982. Meiotic maturation and the cortical granule reaction in starfish eggs. *Biol. Bull.* **163:** 465–476.

MASUI, Y., AND H. J. CLARKE. 1979. Oocyte maturation. *Int. Rev. Cytol.* **57:** 185–282.

NIWA, K., AND M. C. CHANG. 1975. Fertilization of rat eggs *in vitro* at various times before and after ovulation with special reference to fertilization of ovarian oocytes matured in culture. *J. Reprod. Fertil.* **43:** 435–451.

OKAMURA, I., AND H. NISHIYAMA. 1978. Penetration of spermatozoan into the ovum and transformation of the sperm nucleus into the male pronucleus in the domestic fowl, *Gallus gallus*. *Cell Tiss. Res.* **190:** 89–98.

PASTEELS, J. J. 1963. Sur l'origine de la membrane nucleaire du pronucleus chez le mollusque Bivali *Barnea candida* (Etude an microscope electronique). *Bull. Acad. Roy. Sci. (Belgique)* **49:** 329–336.

RAPPAPORT, R. 1971. Cytokinesis in animal cells. *Int. Rev. Cytol.* **31:** 169–213.

RAPPAPORT, R. 1975. Establishment and organization of the cleavage mechanism. Pp. 287–304 in *Molecules and Cell Movement*. S. Inoué and R. E. Stephens, Eds. Raven Press, N. Y.

SCHUETZ, A. W. 1975. Cytoplasmic activation of starfish oocytes by sperm and divalent ionophore (A-23187). *J. Cell Biol.* **66:** 86–94.

SCHUETZ, A. W., AND J. D. BIGGERS. 1967. Regulation of germinal vesicle breakdown in starfish oocytes. *Exp. Cell Res.* **46:** 624–628.

SCHUETZ, A. W., AND F. J. LONGO. 1979. Hormonal control of sperm nuclear decondensation and pronuclear formation in starfish oocytes. *J. Cell Biol.* **83:** 205A.

SCHUETZ, A. W., AND F. J. LONGO. 1981. Hormone-cytoplasmic interactions controlling sperm nuclear decondensation and male pronuclear development in starfish oocytes. *J. Exp. Zool.* **215:** 107–111.

SKOBLINA, M. N. 1974. Behavior of sperm nuclei injected into intact ripening and ripe toad oocytes and into oocytes ripening after removal of the germinal vesicle. *Ontogenez* **5:** 334–340.

SKOBLINA, M. N. 1976. Role of karyoplasm in the emergence of capacity of egg cytoplasm to induce DNA synthesis in transplanted sperm nuclei. *J. Embryol. Exp. Morphol.* **36:** 67–72.

THADANI, V. M. 1979. Injection of sperm heads into immature rat oocyte. *J. Exp. Zool.* **210:** 107–116.

USUI, N., AND R. YANAGIMACHI. 1976. Behavior of hamster sperm nuclei incorporated into eggs at various stages of maturation, fertilization, and early development. *J. Ultrastruct. Res.* **57:** 276–288.

WILSON, E. B. 1925. The Cell in Development and Heredity. Macmillian Co., N. Y.

WITKOWSKA, A. 1981. Pronuclear development and the first cleavage division in polyspermic mouse eggs. *J. Reprod. Fertil.* **62:** 493–498.